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By: Leslie Lindsay

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Rex Bitner *et al.*	Docket No.	016026/9038
Serial No.	09/475,958	Group Art Unit	1655
Confirmation No.	7117	Examiner:	B. Sisson
Filed:	December 30, 1999		

For: CELL CONCENTRATION AND LYSATE CLEARANCE USING PARAMAGNETIC PARTICLES

AMENDMENT AND REQUEST FOR RECONSIDERATION 37 CFR 1.111

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

In the matter of the above-identified application, and in response to the non-final Office Action mailed April 5, 2002, Applicants respectfully request entry of the amendment, reconsideration on the merits of the application, and allowance of the claims.

AMENDMENTS

In the specification:

Please replace the paragraph beginning with "When the silica magnetic particles . . .", on page 11, line 27 of the specification, with the following paragraph:

E1

When the silica magnetic particles have ion exchange ligands covalently attached thereto, the silica-based surface material acts primarily as a solid support for the ion exchange ligands, which enable the particles to form complexes with the various solutes to be isolated or removed from any given solution. When used to isolate a target nucleic acid, the ion exchange ligands are preferably capable of forming a complex with the target nucleic acid by exchanging therewith at one pH, and of releasing the target nucleic acid at another pH. The most preferred ion exchange ligands are ones which complex with the target nucleic acid at a pH which is lower than a neutral pH, and which release the target nucleic acid at about a neutral pH and in low salt conditions, so the target nucleic acid released therein can used immediately, without concentration or further isolation. Such preferred ion exchange ligands and pH dependent ion exchange matrices which incorporate such ligands are described in U.S. Patent Application Ser. No. 09/312,172, now U.S. Patent No. 6,310,199, for an invention titled pH DEPENDENT ION EXCHANGE MATRIX AND METHOD OF USE IN THE ISOLATION OF NUCLEIC ACIDS, incorporated by reference herein, an application filed concurrently with the provisional patent application on which the present non-provisional patent application is based.

In the claims:

Please replace claims 7, 13-16, and 19 with the following claims. Changes to the claims are reflected in a marked-up version of the claims attached to this amendment.

E2

7. A method of clearing a solution of disrupted biological material other than target nucleic acids, according to steps comprising:

(a) providing a solution comprising a disrupted biological material;

(b) combining the solution with magnetic particles under conditions wherein the disrupted biological material other than target nucleic acids selectively adsorbs directly to the particles, thereby forming a complex, wherein said magnetic particles are pH dependent ion exchange particles selected from the group consisting of glycidyl-histidine modified silica magnetic particles, and glycidyl-alanine modified silica magnetic particles; and

(c) separating the complex from the solution by application of magnetic force.

E2 cont.

13. The method of claim 8, wherein the magnetic particles are silica magnetic particles.

14. The method of claim 8, wherein the magnetic particles are pH dependent ion exchange magnetic particles.

E3

15. A method of clearing a solution of disrupted biological material other than target nucleic acids, according to steps comprising:

(a) providing a solution comprising a disrupted biological material;

(b) combining the solution with magnetic particles under conditions wherein the disrupted biological material other than target nucleic acids selectively adsorbs directly to the particles, thereby forming a complex, wherein the magnetic particles are pH dependent ion exchange particles selected from the group consisting of glycidyl-histidine modified silica magnetic particles and glycidyl-alanine modified silica magnetic particles; and

(c) separating the complex from the solution by application of magnetic force.

16. A method of clearing a solution of disrupted biological material other than target nucleic acids, according to the steps comprising:

(a) combining a solution with cells contained therein with first magnetic particles, under conditions wherein the cells selectively adsorb directly to the first magnetic particles;

(b) isolating the complex from the solution by application of magnetic force;

(c) disrupting the cells to provide a solution comprising a disrupted biological material;

(d) combining the solution of step (c) with second magnetic particles under conditions wherein the disrupted biological material other than target nucleic acids

E3 cont

selectively adsorbs directly to the second magnetic particles, thereby forming a complex; and

(e) separating the complex of step (d) from the solution of step (d) by application of magnetic force.

19. A method of clearing a solution of disrupted biological material other than target nucleic acids, according to the steps comprising:

E4

(a) combining a solution with cells contained therein with first pH-dependent ion exchange magnetic particles selected from the group consisting of glycidyl-histidine modified silica magnetic particles, and glycidyl-alanine modified silica magnetic particles, under conditions wherein the cells selectively adsorb directly to the first pH-dependent ion exchange magnetic particles;

(b) isolating the complex from the solution by application of magnetic force;

(c) disrupting the cells to provide a solution comprising a disrupted biological material;

(d) combining the solution of step (c) with second magnetic particles under conditions wherein the disrupted biological material other than target nucleic acids selectively adsorbs directly to the second magnetic particles, thereby forming a complex; and

(e) separating the complex of step (d) from the solution of step (d) by application of magnetic force.

REMARKS

Claims 1-25 and 27-29 are pending in the application. In a non-final Office Action mailed April 5, 2002, the Examiner withdrew the rejection of claims 1-6, 8-18, 20-25, and 27-29 under 35 U.S.C. 102(e) as being anticipated by Smith *et al.* (U.S. Patent No. 6,027,945) and the rejection of claims 8, 9, 18, and 19 under 35 U.S.C. 103(a) as being unpatentable over Smith *et al.* (U.S. Patent No. 6,027,945) and Sosnowski *et al.* (U.S. Patent No. 6,051,380). Further, claims 1-7, 21-25, and 27-29 were rejected under 35 U.S.C. 112, first paragraph; claims 1, 8-15, and 21 were rejected under 35 U.S.C. 112, second paragraph; claims 1, 3, 4, and 6 were rejected under 35 U.S.C. 102(b); claims 1-25 and 27-29 were rejected under 35 U.S.C. 102(f); claims 8-20 were rejected under 35 U.S.C. 102(e); claims 1-7, 21-25, and 27-29 were rejected under 35 U.S.C. 103(a); and claims 8-20 were rejected under the judicially created doctrine of obviousness-type double patenting.

In view of the amendments above and the arguments below, Applicants respectfully request reconsideration on the merits of the application, and allowance of all pending claims.

Objections to the specification

The Examiner has objected to the specification as failing to reflect the current status of U.S. patent applications cited therein. Applicants have amended page 11 of the specification to reflect that U.S. Serial No. 09/312,172 has issued as U.S. Patent No. 6,310,199.

Priority

The Examiner asserted that the provisional application to which the present application claims priority fails to provide adequate support under 35 U.S.C. 112 for claims 1-25 and 27-29. The only explanation offered for stating that claims 1-25 and 27-29 were not supported by the provisional application was a reference to the rejection of claims under 35 U.S.C. 112, first paragraph. However, only claims 1-7, 21-25, and 27-29 were rejected under 35 U.S.C. 112, first paragraph. Because the Examiner has provided no basis for asserting that the provisional application does not provide support for claims 8-20, Applicants are unable to address that determination other than to assert that claims 8-20 are fully supported by U.S. Provisional Application Number 60/134,156 filed May 14, 1999 (hereinafter, "the '156 provisional"). Applicants urge that the claims 1-7, 21-25, and 27-29 of the present invention are fully supported by, and is entitled to the claim of priority to, the '156 provisional for the

reasons set forth herein below in the section entitled "Rejections under 35 U.S.C. 112, first paragraph". Applicants respectfully request that the Examiner acknowledge that the '156 provisional provides support for each of claims 1-25 and 27-29.

Objection to the claims

Claim 7 is objected to under 37 C.F.R. 1.75(c) as being of improper dependent form for failing to further limit the subject matter of claim 1, from which it depends. The Examiner asserts that claim 7, which requires particles comprising glycidyl-histidine or glycidyl-alanine modified silica magnetic particles, actually broadens claim 1. Claim 1 requires that the cells selectively adsorb directly to the particles, whereas in the method of claim 7, the cells would not adsorb directly to the particles. Applicants have rewritten claim 7 in independent form, thereby obviating this objection.

Rejections under 35 U.S.C. 112, first paragraph

Claims 1-7, 21-25, and 27-29 stand rejected under 35 U.S.C. 112, first paragraph for lack of enablement. Citing column 18 (lines 24-27) and Example 9 of Smith *et al.* (U.S. Patent No. 6,319,199 B1), the Examiner asserts that, although the instant specification teaches separation of cells or disrupted biological material, Smith *et al.* "teaches by way of example that these very particles do not bind to proteins but instead bind to DNA." The Examiner argued that the ability of glycidyl-histidine ion exchange particles to selectively bind DNA in a bacterial lysate as disclosed in Example 9 of Smith *et al.*, "is in direct contradiction of the asserted affinity of the same particles in the present application."

Applicants respectfully submit that the method as claimed is fully supported by the specification, and that the disclosure of Smith *et al.* does not contradict the asserted affinity of the same particles in the present application. Contrary to the Examiner's characterization of Smith *et al.*, Example 9 of Smith *et al.* does not disclose selective adsorption of DNA from a bacterial lysate to particles. Rather, Example 9 shows binding of DNA from a cleared bacterial lysate, prepared according to Example 6, in which disrupted biological material other than target nucleic acids had been removed from the lysate by centrifugation prior to combining with the particles.

The claims of the instant application require combining the particles and the material to be adsorbed to the particles under conditions wherein the material selectively adsorbs to the particles to form a complex. In contrast to Smith *et al.*, claims 21-25 and 27-29 require first contacting disrupted biological material with particles under conditions in which the disrupted biological material other than the target nucleic acid adsorbs to particles to form a cleared solution, and subsequently contacting the cleared solution with particles under conditions in which the target nucleic acid adsorbs to the particles.

The conditions used to isolate cells according to the method of claims 1-7 differ from conditions used to isolate target DNA according to Smith *et al.* For example, compare conditions described at page 10, lines 10-11 and Example 8 of the instant application with Example 9 of Smith *et al.* Contrary to the Examiner's assertion that Smith *et al.* "is in direct contradiction of the asserted affinity of the same particles in the present application", the specification of the subject application discloses that different conditions may affect binding affinities of particles and describes conditions suitable for isolating cells. Therefore, Smith *et al.* does not contradict the asserted affinity of the same particles in the present application. Applicants respectfully request that the rejection under 35 U.S.C. 112, first paragraph be withdrawn.

Rejection of the claims under 35 U.S.C. 112, second paragraph

The Examiner has rejected claims 1, 8, and 21 as being indefinite because dependent claims 7, 15, and 19 include a glycidyl-histidine or glycidyl-alanine moiety on the surface of the particles, and "effectively broadens the scope of the claims and allows for the claims to be interpreted as permitting virtually any molecule to be bound thereto." Claims 7, 15, and 19 have been rewritten in independent form and no longer depend from claims 1 and 8. Therefore, the scope of claim 1 or 8 is not broadened by claims 7, 15, or 19. Because none of the claims depending from claim 21 require a glycidyl-histidine or glycidyl-alanine moiety on the surface of the particles, Applicants submit that claim 21 is not broadened by any dependent claim and is not indefinite under the basis for this rejection given by the Examiner. Accordingly, Applicants respectfully request that the rejection be withdrawn.

Independent claim 8, and claims 9-15, which depend from claim 8, stand rejected as being indefinite for the recitation of 'second magnetic particles', which expression lacks an antecedent basis. The rejection was made in the previous Office Action, and with respect to

claims 8-12, we believe should have been overcome by entry of the amendment filed November 5, 2001, in which the word "second" was deleted from claim 8. Claims 13-15, which also recite "second magnetic particles", have been amended to delete the word "second". Applicants respectfully request that the rejection be withdrawn.

For the sake of clarity, Applicants have rewritten claim 16 as an independent claim to provide antecedent basis for "first magnetic particles", as recited in claims 16-20.

Rejections under 35 U.S.C. 102

Rejections under 35 U.S.C. 102(b)

Claims 1, 3, 4, and 6 stand rejected under 35 U.S.C. 102(b) as being anticipated by Margel (US Patent 4,861,705). Margel is characterized as disclosing "agarose particles that can be configured as an ion exchange resin as well as being magnetic." The Examiner asserts Margel teaches that the agarose particles may be used in affinity chromatography and cell separation, that the particles are compatible with blood, and that magnetic beads having an amino-spacer bound thereto may be used to separate blood cells. Margel does not teach cells selectively adsorbing directly to magnetic particles to form a complex, as required by claims 1, 3, 4, and 6. Rather, Margel teaches beads comprising polyacrolein or polyglutaraldehyde microspheres encapsulated in agarose, which may also contain magnetic particles. Example 35 describes making "immunobeads" by reacting goat anti-rabbit IgG with beads comprising a spacer of polylysine-glutaraldehyde. The immunobeads were then used to separate human RBCs sensitized with rabbit anti-human RBC antibodies from turkey RBCs. Clearly, the cells are complexed with particles through binding of antibodies, which are complexed with aldehydes on the particles, to antibodies adsorbed to the human RBCs sensitized with rabbit anti-human antibodies. Margel does not teach cells selectively adsorbing directly to the particles, as required by claims 1, 3, 4, and 6.

Rejections under 35 U.S.C. 102(f)

The Examiner has rejected claims 1-25 and 27-29 under 35 U.S.C. 102(f) because the "applicant did not invent the claimed subject matter." The present application and Smith *et al.* have inventors in common, but have different inventive entities. The Examiner also asserts that because the assignment was recorded with the USPTO after the application was filed, the application does not appear to have been commonly owned at the time of filing, and

Smith *et al.* is available as prior art under 35 U.S.C. 102(f). The Examiner asserts that claims 1-25 and 27-29 require mixing pH-dependent ion exchange particles or silica magnetic particles with either cells or cell membranes, and that Smith *et al.* (U.S. Patent No. 6,319,199 B1) discloses just such steps.

The Examiner has provided no basis in fact for the assertion that the present inventors derived the invention as claimed from the inventors named on U.S. Patent No. 6,319,199 B1. Each inventor named on the present application has executed a declaration stating that he believes he is "an original, first and joint inventor of the subject matter which is claimed and for which a patent is sought". The present application is entitled to the claim of priority to the provisional application, which was filed May 14, 1999, the same day that the application giving rise to U.S. Patent No. 6,319,199 B1 was filed. Therefore, Smith *et al.* is not available as prior art, and there is no basis for presuming that the inventors of the instant application derived the claimed invention from the named inventors on Smith *et al.*

Furthermore, the present invention is not anticipated by Smith *et al.* because Smith *et al.* fails to teach all of the claim limitations. The Examiner asserts the claims require mixing pH-dependent ion exchange particles or silica magnetic particles with either cells or cell membranes, and that Smith *et al.* (U.S. Patent No. 6,319,199 B1) discloses just such steps. Applicants are confused by the rejection of claims on this basis, particularly in view of the Examiner's rejection of claims under 35 U.S.C. 112, first paragraph based on the assertion that Smith *et al.*, "is in direct contradiction of the asserted affinity of the same particles in the present application." Smith *et al.* focused primarily on isolating nucleic acids from disrupted cells using silica magnetic particles under conditions that favor adsorption of nucleic acids to the particles. In contrast, the claims of the present invention are directed toward isolating cells using conditions under which cells selectively adsorb to the particles, or clearing biological material other than target nucleic acids from disrupted biological material using conditions under which disrupted biological material other than target nucleic acids selectively adsorb to the particles.

Rejections under 35 U.S.C. 102(e)

Claims 8-20 stand rejected under 35 U.S.C. 102(e) as being anticipated by Smith *et al.* (US Patent 6,310,199 B1). The Examiner asserts that Smith *et al.* discloses using "these very

particles in a method whereby biological material is cleared of nucleic acid sequences". Because Smith *et al.* was filed the same day as the priority date of the present application, it is not available as prior art.

Even if Smith *et al.* were available as prior art, it does not anticipate the claimed invention, because it does not teach the limitation of combining the particles with disrupted biological material under conditions that cause the disrupted biological material other than target nucleic acid to selectively adsorb directly to the particles. In fact, the Examiner expressed skepticism as to whether the methods as claimed were enabled, asserting that Smith *et al.*, "is in direct contradiction of the asserted affinity of the same particles in the present application". Applicants respectfully submit that the claims of the instant application are not anticipated by Smith *et al.*

Rejections under 35 U.S.C. 103(a)

Claims 1-7, 21-25, and 27-29 stand rejected under 35 U.S.C. 103(a) as being unpatentable over Nelson *et al.* (U.S. Patent No. 6,344,326 B1) in view of Margel (U.S. Patent No. 4,861,705) and Smith *et al.* (U.S. Patent No. 6,310,199 B1) taken with Smith *et al.* (U.S. Patent No. 6,027,945).

Nelson *et al.*, column 19, is cited as disclosing isolating one or more components from a solution using a first and second capture agent. The Examiner acknowledges that Nelson does not disclose the specific particles of the claimed invention.

Margel is cited as disclosing "agarose particles that can be configured as an ion exchange resin as well as being magnetic," which the Examiner asserts may be used in affinity chromatography and cell separation. The Examiner further states that the particles are compatible with blood, and that magnetic bead having an amino-spacer bound thereto may be used to separate blood cells. The Examiner acknowledges that Margel does not teach using silica magnetic particles for isolating cells.

Smith *et al.* (U.S. Patent No. 6,310,199) is cited as disclosing a method whereby a target nucleic acid is isolated from disrupted biological material using the particles recited in the claims. The Examiner fails to elaborate on the putative significance of Smith *et al.* (U.S. Patent No. 6,027,945).

The Examiner concluded that it would have been obvious to modify the procedure of Nelson *et al.* with the methods of Margel, Smith *et al.* and Smith *et al.* and to use a first population of particles to clear a solution of cell membranes and the like from a solution (Margel) and to use a second populations of particles to isolate nucleic acids (Smith *et al.* and Smith *et al.*).

A *prima facie* case of obviousness requires: (1) some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings; (2) a reasonable expectation of success; and (3) the art reference or combination of references must teach all of the claim limitations (MPEP 2142).

Applicants note that Nelson *et al.* was filed on February 10, 2000, after the filing date and the priority date of the instant application, and is therefore not available as prior art. The disclosure of Nelson *et al.* relates primarily to using microfluidic devices having affinity-capture microchannels comprising immobilized receptors such as antibodies specific for cell surface antigens to capture biological entities, including whole cells. Nelson et. al discusses at column 19 contacting a sample of a mixed population of biological entities with a plurality of capture agents under conditions favoring specific binding of different capture agents to different subsets of biological entities.

Margel does not teach cells selectively adsorbing directly to magnetic particles to form a complex, as required by claims 1, 3, 4, and 6. Rather, Margel teaches beads comprising polyacrolein or polyglutaraldehyde microspheres encapsulated in agarose, which may also contain magnetic particles. The Abstract discloses that the polyaldehyde beads bind in a single step through the aldehyde groups to compounds containing primary amino groups or thiol groups, such as proteins, antibodies, enzymes, and drugs. Example 35 describes making "immunobeads" by reacting goat anti-rabbit IgG with beads comprising a spacer of polylysine-glutaraldehyde. The immunobeads were then used to separate human RBCs sensitized with rabbit anti-human RBC from turkey RBCs.

Applicants assert that Smith *et al.* (U.S. Patent No. 6,310,199 B1) is not available as prior art. Neither of the Smith *et al.* patents cited by the Examiner disclose concentrating or harvesting cells, as required by claims 1-7. Neither patent contemplates using particles to

clear a solution of disrupted biological material of non-target material, as required by claims 21-25 and 27-29.

Applicants respectfully submit that the Examiner has not established prima facie case of obviousness because Nelson *et al.*, Margel, and the two Smith *et al.* patents do not combine to teach all of the claim limitations of claims 1-7, 21-25, or 27-29. Both Nelson *et al.* and Margel teach using antibodies to capture or sort cells. The Examiner acknowledges that neither Nelson *et al.* nor Margel teach using the particles recited in claims 1-7, 21-25, or 27-29. Each of the Smith *et al.* publications cited is silent as to the use of the particles in concentrating or harvesting cells. Therefore, nothing in the art of record suggests concentrating or harvesting cells using pH dependent ion exchange particles or silica magnetic particles under conditions wherein the cells selectively adsorb to the particles. one of skill in the art would not be motivated to modify Nelson *et al.* or Margel *et al.* to use. Applicants acknowledge that desire to isolate or concentrate cells predates their invention. However, nothing in the art of record teaches or suggests the Applicants' method, as recited in claims 1-7.

Double Patenting Rejections

The Examiner has rejected claims 8-20 under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 37, 38, 41, 45, 52-54, 56, and 57 of U.S. Patent No. 6,310,199 B1. Claims 8-20 of the instant application are not obvious over claims 37, 38, 41, 45, 52-54, 56, and 57 of U.S. Patent No. 6,310,199 B1. The claims identified by the Examiner involve isolating a target nucleic acid by adsorbing target nucleic acid to a pH dependent ion exchange matrix at a first pH and desorbing the target nucleic acid at a second pH. In contrast, claims 8-20 recite a method of clearing a solution of disrupted biological material other than target nucleic acid involving combining the solution with magnetic particles under conditions wherein the disrupted biological material other than target nucleic acid selectively adsorb to the particles. In the case of claims 37, 38, 41, 45, 52-54, 56, and 57 of Smith *et al.*, the target nucleic acid adsorbs to the pH dependent ion exchange matrix. In contrast, in the present invention, disrupted biological material other than the target nucleic acid selectively adsorbs to silica magnetic particles or pH-dependent ion exchange particles.

Claims 8-20 stand rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-29 of U.S. Patent No. 6,027,945 "because both encompass isolation of nucleic acids from disrupted biological material." Applicants acknowledge that claims 1-29 of Smith *et al.* encompass isolating target biological material (e.g., target nucleic acids). However, in the case of claims 1-29 of Smith *et al.*, the target material is isolated by adhering the target material to silica magnetic particles followed by desorbing the target material from the particles. In contrast, in the methods of claims 8-20, a solution of disrupted biological material is cleared by combining magnetic particles with the solution of disrupted biological material under conditions wherein disrupted biological material other than target nucleic acids selectively adsorbs to the particles. Claims 8-20 of the instant application are not unpatentable over claims 1-29 of U.S. Patent No. 6,027,945 because the art does not teach or suggest all of the claim limitations.

Applicants respectfully submit the a prima facie case of obviousness has not been established because the claims do not combine to teach all of the claim limitations. Applicants request that the rejection under the judicially created doctrine of obviousness-type double patenting be withdrawn.

No fee is believed due in connection with this response. However, if a fee is owed, please charge such fee to Deposit Account No. 50-0842.

Respectfully submitted,

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